Document Number 17

Entry 17 of 17 File: DWPI Dec 8, 1998

DERWENT-ACC-NO: 1999-045198
DERWENT-WEEK: 199916

TITLE: Screening for compounds to treat impaired glucose tolerance conditions - comprises use of DAF polypeptides functionally complementing C. elegans daf mutation or having identity to C. elegans polypeptide
INVENTOR: KIMURA, K; KOWEEK, A ; MORRIS, J ; OGG, S ; PARADIS, S ; PATTERSON, G ; PIERCE, S ; RUVKUN, G ; TISSENBAUM, H

PRIORITY-DATA:

1997US-0888534	July 7, 1997
1997US-0857076	May 15, 1997

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9874941 A	December 8, 1998	N/A	000	A61K049/00
WO 9851351 A1	November 19, 1998	E	202	A61K049/00

INT-CL (IPC): A61K 49/00; C07H 21/04; C12N 5/06

ABSTRACTED-PUB-NO: WO 9851351A
BASIC-ABSTRACT:

The following are claimed: (1) a method of screening for a compound that decreases activity of a DAF polypeptide comprises: (a) exposing non-human transgenic animal, whose cells comprise a transgene coding for a mammalian DAF polypeptide to compound, and (b) determining activity of DAF polypeptide in animal, a decrease indicating compound decreasing DAF activity; (2) identifying compounds decreasing expression/a ctivity of a daf gene, by contacting cell expressing daf gene with test compound and observing decrease in daf expression/activity; (3) identifying compounds ameliorating or delaying an impaired glucose tolerance condition, by: (i) contacting dauer larvae comprising mutation in daf gene with test compound and observing release from dauer state; or (ii) expressing in cells of a daf-2, daf-16 or a daf-7, daf-3 mutant nematode a mammalian DAF-16 or DAF-3 polypeptide respectively, whereby nematode forms dauer larva, contacting with test compound and observing release from dauer state; (4) determining if human gene is involved in impaired glucose tolerance condition or obesity, by expressing gene operatively linked to nematode gene promoter in nematode

having daf or age gene mutation, and observing complementation of daf or age mutation; (5) diagnosing impaired glucose tolerance condition or obesity (or propensity to) in mammals by detecting mutation in DAF gene in DNA, and (6) DNA encoding DAF-16 polypeptide and complementing DAF-16 mutation in C. elegans (and optionally complementing FKHR or AFX mutation in mice).

USE - The screening method or methods of (1) or (2) can be used to identify compounds which are useful to ameliorate or delay an impaired glucose tolerance condition (e.g. diabetes or atherosclerosis) or obesity (claimed). The methods (1) or (2) may also be used to specifically identify compounds decreasing expression or activity of DAF-3 or DAF-16, also useful as above (claimed). Such compounds (e.g. DAF polypeptides (especially nematode/human DAF-7 (claimed), anti-diabetic or anti-obesity pharmaceuticals) can then be administered therapeutically to treat such conditions. Method (5) is useful in diagnosis of the existence or propensity to such conditions in humans. The methods and sequence are based on the discovery that DAF polypeptides are involved in glucose metabolism; C. elegans daf genes were found to be excellent candidate genes and proteins for human diseases associated with glucose intolerance, and results indicated that human homologues of these daf genes and proteins mediate insulin signalling in normal people and may be defective or mis-regulated in diabetes.

(FILE 'HOME' ENTERED AT 18:07:40 ON 20 MAR 2001)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 18:09:02 ON 20 MAR 2001

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L1          2482 S DAF-18 OR PTEN
L2            22 S L1 AND COMPOUND
L3            16 DUP REM L2 (6 DUPLICATES REMOVED)
L4            16 SORT L3 PY
L5            61 S L1 AND ELEGANS
L6            23 DUP REM L5 (38 DUPLICATES REMOVED)
L7            23 SORT L6 PY
L8          2462 S PTEN
L9           589 S L8 AND MAMMAL?
L10            9 S L9 AND (GLUCOSE OR DIABET? OR OBES?)
L11            5 DUP REM L10 (4 DUPLICATES REMOVED)
L12            5 SORT L11 PY
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=> d ti so au ab pi l12 1-5

L12 ANSWER 1 OF 5 MEDLINE

TI The C. elegans **PTEN** homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway.

SO MOLECULAR CELL, (1998 Dec) 2 (6) 887-93.
Journal code: C5E. ISSN: 1097-2765.

AU Ogg S; Ruvkun G

AB An insulin-like signaling pathway, from the DAF-2 receptor, the AGE-1 phosphoinositide 3-kinase, and the AKT-1/AKT-2 serine/threonine kinases to the DAF-16 Fork head transcription factor, regulates the metabolism, development, and life span of Caenorhabditis elegans. Inhibition of daf-18 gene activity bypasses the normal requirement for AGE-1 and partially bypasses the need for DAF-2 signaling. The suppression of age-1 mutations by a daf-18 mutation depends on AKT-1/AKT-2 signaling, showing that DAF-18 acts between AGE-1 and the AKT input to DAF-16 transcriptional regulation. daf-18 encodes a homolog of the human tumor suppressor **PTEN** (MMAC1/TEP1), which has 3-phosphatase activity toward phosphatidylinositol 3,4,5-trisphosphate (PIP3). DAF-18 **PTEN** may normally limit AKT-1 and AKT-2 activation by decreasing PIP3 levels. The action of daf-18 in this metabolic control pathway suggests that **mammalian** **PTEN** may modulate insulin signaling and may be variant in **diabetic** pedigrees.

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L12 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2001 ACS

TI Genes and polypeptides involved in insulin signaling pathways for **glucose** tolerance, **obesity**, and longevity and their uses as therapeutic and diagnostic tools

SO PCT Int. Appl., 402 pp.
CODEN: PIXXD2

L7 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2001 ACS
TI Genes and polypeptides involved in insulin signaling pathways for glucose tolerance, obesity, and longevity and their uses as therapeutic and diagnostic tools
SO PCT Int. Appl., 402 pp.
CODEN: PIXXD2
IN Ruvkun, Gary; Ogg, Scott
AB Disclosed herein are novel genes and methods for the screening of therapeutics useful for treating impaired glucose tolerance conditions, as well as diagnostics and therapeutic compns. for identifying or treating such conditions. The Caenorhabditis **elegans** metabolic regulatory genes daf-2 and age-1 encode homologs of the mammalian insulin receptor/phosphoinositol 3-kinase signaling pathway proteins, resp. Also, the C. **elegans** PKB kinase and AKT kinase act downstream of these genes, as their mammalian homologs act downstream of insulin signaling. The C. **elegans PTEN** lipid phosphatase homolog, **DAF-18**, acts upstream of AKT in this signaling pathway. Further, the DAF-16 forkhead protein represents the major transcriptional output of this insulin signaling pathway. Addnl. evidence indicates that the DAF-16, DAF-3, DAF-8, and DAF-14 transcriptional outputs of converging signaling pathways regulate metab. The congruence between the C. **elegans** and mammalian insulin signaling pathways strongly supports the contention that new genes identified in the C. **elegans** pathway also act in mammalian insulin signaling. Exemplary sequences and functional characteristics of the C. **elegans** daf genes and their human homologs are provided.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000033068	A1	20000608	WO 1999-US28529	19991202

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

L7 ANSWER 23 OF 23 BIOSIS COPYRIGHT 2001 BIOSIS
TI The **PTEN** tumor suppressor protein: An antagonist of phosphoinositide 3-kinase signaling.
SO Biochimica et Biophysica Acta., (Feb. 14, 2000) Vol. 1470, No. 1, pp. M21-M35.
ISSN: 0006-3002.
AU Vazquez, Francisca; Sellers, William R. (1)

L7 ANSWER 11 OF 23 MEDLINE

TI Regulation of the insulin-like developmental pathway of Caenorhabditis **elegans** by a homolog of the **PTEN** tumor suppressor gene.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Mar 16) 96 (6) 2925-30.
Journal code: PV3. ISSN: 0027-8424.

AU Gil E B; Malone Link E; Liu L X; Johnson C D; Lees J A

AB The human **PTEN** tumor suppressor gene is mutated in a wide variety of sporadic tumors. To determine the function of **PTEN** in vivo we have studied a **PTEN** homolog in Caenorhabditis **elegans**. We have generated a strong loss-of-function allele of the **PTEN** homolog and shown that the deficient strain is unable to enter dauer diapause. An insulin-like phosphatidylinositol 3-OH kinase (PI3'K) signaling pathway regulates dauer-stage entry. Mutations in either the daf-2 insulin receptor-like (IRL) gene or the age-1 encoded PI3'K catalytic subunit homolog cause constitutive dauer formation and also affect the life span, brood size, and metabolism of nondauer animals. Strikingly, loss-of-function mutations in the age-1 PI3'K and daf-2 IRL genes are suppressed by loss-of-function mutations in the **PTEN** homolog. We establish that the **PTEN** homolog is encoded by **daf-18**, a previously uncloned gene that has been shown to interact genetically with the DAF-2 IRL AGE-1 PI3'K signaling pathway. This interaction provides clear genetic evidence that **PTEN** acts to antagonize PI3'K function in vivo. Given the conservation of the PI3'K signaling pathway between C. **elegans** and mammals, the analysis of **daf-18 PTEN** mutant nematodes should shed light on the role of human **PTEN** in the etiology of metabolic disease, aging, and cancer.

L7 ANSWER 10 OF 23 MEDLINE

TI Regulation of dauer larva development in Caenorhabditis **elegans** by **daf-18**, a homologue of the tumour suppressor **PTEN**.

SO CURRENT BIOLOGY, (1999 Mar 25) 9 (6) 329-32.
Journal code: B44. ISSN: 0960-9822.

AU Rouault J P; Kuwabara P E; Sinilnikova O M; Duret L; Thierry-Mieg D; Billaud M

AB The tumour suppressor gene **PTEN** (also called MMAC1 or TEP1) is somatically mutated in a variety of cancer types [1] [2] [3] [4]. In addition, germline mutation of **PTEN** is responsible for two dominantly inherited, related cancer syndromes called Cowden disease and Bannayan-Ruvalcaba-Riley syndrome [4]. **PTEN** encodes a dual-specificity phosphatase that inhibits cell spreading and migration partly by inhibiting integrin-mediated signalling [5] [6] [7]. Furthermore, **PTEN** regulates the levels of phosphatidylinositol 3,4,5-trisphosphate (PIP3) by specifically dephosphorylating position 3 on the inositol ring [8]. We report here that the dauer formation gene **daf-18** is the Caenorhabditis **elegans** homologue of **PTEN**. **DAF-18** is a component of the insulin-like signalling pathway controlling entry into diapause and adult longevity that is regulated by the DAF-2 receptor tyrosine kinase and the AGE-1 PI 3-kinase [9]. Others have shown that mutation of **daf-18** suppresses the life extension and constitutive dauer formation associated with daf-2 or age-1 mutants. Similarly, we show that inactivation of **daf-18** by RNA-mediated interference mimics this suppression, and that a wild-type **daf-18** transgene rescues the dauer defect. These results indicate that **PTEN/daf-18** antagonizes the DAF-2-AGE-1 pathway, perhaps by catalyzing dephosphorylation of the PIP3 generated by AGE-1. These data further support the notion that mutations of **PTEN** contribute to the development of human neoplasia through an aberrant activation of the PI 3-kinase signalling cascade.

L7 ANSWER 9 OF 23 MEDLINE

TI The **PTEN** tumor suppressor homolog in Caenorhabditis **elegans** regulates longevity and dauer formation in an insulin receptor-like signaling pathway.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Jun 22) 96 (13) 7427-32.
Journal code: PV3. ISSN: 0027-8424.

AU Mihaylova V T; Borland C Z; Manjarrez L; Stern M J; Sun H

AB Inactivation of the tumor suppressor **PTEN** gene is found in a variety of human cancers and in cancer predisposition syndromes. Recently, **PTEN** protein has been shown to possess phosphatase activity on phosphatidylinositol 3,4,5-trisphosphate, a product of phosphatidylinositol 3-kinase. We have identified a homolog of **PTEN** in Caenorhabditis **elegans** and have found that it corresponds to the **daf-18** gene, which had been defined by a single, phenotypically weak allele, **daf-18** (e1375). By analyzing an allele, **daf-18**(nr2037), which bears a deletion of the catalytic portion of CePTEN/**DAF-18**, we have shown that mutation in **daf-18** can completely suppress the dauer-constitutive phenotype caused by inactivation of daf-2 or age-1, which encode an insulin receptor-like molecule and the catalytic subunit of phosphatidylinositol 3-kinase, respectively. In addition, **daf-18**(nr2037) dramatically shortens lifespan, both in a wild-type background and in a daf-2 mutant background that normally prolongs lifespan. The lifespan in a **daf-18**(nr2037) mutant can be restored to essentially that of wild type when combined with a daf-2 mutation. Our studies provide genetic evidence that, in C. **elegans**, the **PTEN** homolog **DAF-18** functions as a negative regulator of the DAF-2 and AGE-1 signaling pathway, consistent with the notion that **DAF-18** acts a phosphatidylinositol 3,4,5-trisphosphate phosphatase in vivo. Furthermore, our studies have uncovered a longevity-promoting activity of the **PTEN** homolog in C. **elegans**.